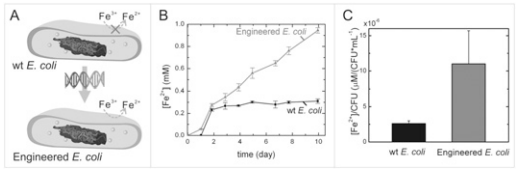
1988-Plat
Growing Electrical Connections in Living Cells
Heather M. Jensen[1], Jay T. Groves[2], Caroline M. Ajo-Franklin[3].
[1]UC Berkeley/Lawrence Berkeley National Lab, Berkeley, CA, USA, [2]UC Berkeley, Berkeley, CA, USA, [3]Lawrence Berkeley National Lab, Berkeley, CA, USA.
Cellular-electrical connections have the potential to combine the specialties of the technological world with those of the living world. However, cell membranes are natural insulators, inherently creating a barrier between intracellular electrons and inorganic materials. To overcome this barrier, we have 'grown' electrical connections in living cells by engineering the cell to construct a well-defined electron pathway. The dissimilatory metal-reducing microbe, *Shewanella oneidensis* MR-1, inspired our approach: it has the unusual ability to transport electrons to extracellular minerals via a transmembrane electron transport pathway (ETP). We seek to generalize this ability to grow electrical contacts between microbes and inorganic materials, and thus have genetically re-engineered a portion of the *Shewanella* ETP into *Escherichia coli* as a first step towards building microbial-electrical interfaces (Fig 1A). Native *E. coli* proteins complete the electron pathway by directly interacting with the functionally expressed *Shewanella* proteins. These 'electrified' strains exhibit ~8x and ~4x faster the metal reducing efficiency with soluble metals and insoluble metal oxides, respectively, than wild-type *E. coli* (Fig. 1B,C). These experiments provide the first steps towards engineering of hybrid living-nonliving systems.



1989-Plat
Culturing Human Primary Lung Cells at the Air-Media Interface with Magnetic Levitation
Glauco Souza[1], Dan Stark[2], Carly Figueria[1], **Robert Raphael**[2], Tom Killian[2].
[1]Nano3D Biosciences, Houston, TX, USA, [2]Rice University, Houston, TX, USA.
Improved methods for producing three dimensional cultures of human lung cells are needed for lung tissue engineering and drug discovery. We have cultured normal endothelial, epithelial, fibroblast, and smooth muscle human primary lung cells (ScienCell Research Laboratories) at the air-media interface using magnetic levitation, a new method for three-dimensional cell culture. Magnetic levitation relies upon a mixture of gold and magnetic iron oxide nanoparticles and biocompatible polymers to deliver magnetic nanoparticles to cells and strong magnetic fields to lift cells of the bottom of the petri dish and culture them at the air-liquid interface. It allows cells to grow in three-dimensions, which is of significant interest because, for many applications, three-dimensional culturing provides a better representation of the in vivo environment than traditional two-dimensional cell culture, and it produces tissue that is more representative of natural morphology, protein expression, and response to drugs. Cells can be cultured at the air-liquid interface, which is particularly advantageous for specialized tissue such as lung. Bright field microscopy images are used to study overall cell morphology, and we observe characteristic structures typically attributed to respective cell types, such as the formation of squamous structure for epithelial cells. The production of extracellular matrix proteins such as collagen can be detected using van Gieson's staining. The magnetic levitation method will be further developed to manipulate tissue shape in mono and co-cultures of lung cells. (Funded by NSF Grant# IIP 0945954)

1990-Plat
Tunable Morphology and Structural Properties of Recombinant Silk-Elastinlike Biopolymers by Electrospinning
Raul Machado[1], Carmen García-Arévalo[2], Francisco Javier Arias[2,3], José Carlos Rodríguez-Cabello[2,3], Margarida Casal[1].
[1]CBMA - University of Minho, Braga, Portugal, [2]Bioforge - University of Valladolid, Valladolid, Spain, [3]CIBER-BBN, Valladolid, Spain.
Silk-elastinlike proteins (SELPs) are a new class of bioinspired, biologically synthesized block copolymers, consisting in silk fibroin (GAGAGS) and elastin (VPGVG) repeating units. With the aim of developing new high performance protein polymers, we report the electrospinning of two new SELP copolymers based on silk fibroin crystalline blocks and elastin-like thermoplastic (VPAVG) blocks. These new copolymers, named S10E20 and S5E10, where S corresponds to the number of repetitions of the silk block and E to the number of elastin blocks, were chemically synthesized by recombinant DNA technology and biologically produced by *Escherichia coli*. Due to its easy implementation, electrospinning has received a lot of attention as a technique to produce nanofibers. Electrospinning of different SELPs concentrations (5, 9, 13, 17 and 21 wt%) was performed in aqueous solution or in formic acid without addition of external agents. Electrospun structures were analyzed by scanning electron microscopy and the average diameter was calculated. The effect of methanol in the electrospun mat was also evaluated, morphologically by scanning electron microscopy and structurally by analyzing the secondary structure by FTIR. By varying the concentration, the morphology and size of the electrospun structures, can be customized to tailored applications. Polymer concentration and solvent, either water or formic acid, showed to play an important and determinant role in the process of electrospinning. While low concentrations of polymer solution lead to the formation of nano-microsized structures, higher polymer concentrations produced electrospun fibers with increasing diameter and size distribution, ranging from the nano to the sub-microscale. Comparing the solvents, electrospun fibers in aqueous solution lead to the formation of fibers with higher diameter and size distribution.
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Platform AU: Cardiac Muscle II

1991-Plat
Cardiac Myosin Light Chain Kinase is Essential for Myosin Regulatory Light Chain Phosphorylation and Normal Cardiac Function *in vivo*
Peiguo Ding, Jian Huang, Pavan K. Battiprolu, Joseph A. Hill, Kristine E. Kamm, James T. Stull.
University of Texas Southwestern Medical Center, Dallas, TX, USA.
In contrast to studies on skeletal and smooth muscles, protein kinases that are important physiologically for direct phosphorylation of myosin regulatory light chain (RLC) in the heart are not known. Expression of a Ca^{2+}/calmodulin-activated myosin light chain kinase found only in cardiac muscle (cMLCK) was ablated in mice. The extent of RLC phosphorylation was dependent on the extent of cMLCK expression in both ventricular and atrial muscles. Lack of cMLCK and RLC phosphorylation led to (1) ventricular hypertrophy demonstrated by increased heart weight to tibial length ratios, (2) myocyte hypertrophy and (3) histological evidence of necrosis and fibrosis. Loss of MLC2v phosphorylation led to compromised cardiac function evidenced by echocardiography showing a proportional decrease in systolic performance assessed as percent fractional shortening from 71% in wildtype mice to 34% for hearts with no cMLCK. Declines in cardiac function were associated with ventricular dilation and progressive increases in left ventricular end-systolic and end-diastolic dimensions. Hearts from female mice showed similar responses to loss of cMLCK including diminished RLC phosphorylation and significant ventricular hypertrophy. Prolonged isoproterenol infusion elicited hypertrophic cardiac responses in wildtype mice. In mice lacking cMLCK, the hypertrophic hearts showed no additional increases in size with the isoproterenol treatment, suggesting a lack of RLC phosphorylation blunted the stress response. Thus, cMLCK appears to be the predominant protein kinase that maintains basal RLC phosphorylation which is required for normal physiological cardiac performance *in vivo.* Supported by NIH NHLBI.

1992-Plat
Kinetic Analysis of Human α and β Cardiac Myosin Motor Domains
John C. Deacon[1], Marieke J. Bloemink[2], Michael A. Geeves[2], Leslie A. Leinwand[1].
[1]University of Colorado, Boulder, Boulder, CO, USA, [2]University of Kent, Canterbury, United Kingdom.
We report the first kinetic analysis of recombinant human and cardiac myosin subfragments-1 (S1). The two isoforms show significant differences; the kinetics of recombinant β-S1 are similar to β-S1 from mammalian tissues whereas α-S1 has more in common with fast skeletal muscle myosins. The second order rate constant of ATP binding is ~twice as fast for α-S1